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First Molecular Evidence of *Anaplasma ovis* and *Rickettsia* spp. in Keds (Diptera: Hippoboscidae) of Sheep and Wild Ruminants

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Abstract

To evaluate the presence of rickettsial agents in hippoboscid flies with molecular methods, 81 sheep keds (*Melophagus ovinus*) were collected from 23 sheep, 144 deer keds (*Lipoptena cervi*) were caught in the environment, and a further 463 and 59 individuals of the latter species were obtained from fresh carcasses of 29 red deer and 17 roe deer, respectively. DNA was extracted individually or in pools. *Anaplasma ovis* was demonstrated in all examined sheep keds, and from one pool of free-living deer keds. *Rickettsia helvetica* or other, unidentified rickettsiae were also present in one pool of sheep keds, and in four pools of deer keds from both red deer and roe deer. This is the first account of polymerase chain reaction positivity of hippoboscid flies for *A. ovis* and rickettsiae. These results raise the possibility that—apart from cattle and roe deer as already reported—sheep and red deer might also play a reservoir role in the epidemiology of rickettsioses.

Key Words: *Anaplasma ovis*—Hippoboscidae—*Rickettsia helvetica*.

Keds or louse/forest flies (Diptera: Hippoboscidae) are blood-sucking ectoparasites of domestic and wild animals. Although having preference for a certain host species, they are not strictly host specific, because (depending on their species) they may feed on a broader range of animals, even humans (Bequaert 1942, Small 2005). In this way, when they transfer between and suck blood on a succession of host individuals—either of the same or different species—they may carry over bartonellae (Halos et al. 2004). To the best of our knowledge, no reports have been published on the molecular investigation of keds for representatives of the order Rickettsiales. This study was undertaken to evaluate large numbers of hippoboscid flies from various sources for the presence of emerging, potentially zoonotic pathogens: *Anaplasma ovis*, *Rickettsia helvetica,* and other rickettsiae (Fournier et al. 2000, Chochlakis et al. 2010).

Eighty-one adult sheep keds (*Melophagus ovinus*) were removed from 23 sheep with a history of anaplasmosis in northeastern Hungary. Altogether, 144 specimens of deer keds (*Lipoptena cervi*) were collected from the environment manually—while they attempted to suck blood on humans—on different locations in north Hungary. A further 463 and 59 wingless individuals of the latter species were obtained from fresh carcasses of 29 red deer (*Cervus elaphus*) and 17 roe deer (*Capreolus capreolus*), respectively, from various places of the country. Specimens were preserved in 70% ethanol until evaluation.

Sheep keds were processed individually. Free-living deer keds were pooled according to place of collection, with up to three flies per pool. A maximum of 10 host-derived deer keds were grouped together from the same animal. DNA extraction, evaluation of amplifiable DNA, and molecular analyses were done as described previously (Hornok et al. 2010). Together with phosphate-buffered saline extraction controls 184 samples were tested. In brief, conventional polymerase chain reaction (PCR) was performed to screen for the presence of the 16S rRNA gene of various members of the family Anaplasmataceae, then, with the positive samples, for the *msp4* gene of

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Table 1. Molecular Investigation of Keds for Some Representatives of the Order Rickettsiales

	Anaplasma spp: msp4 PCR positives/all tested	*Sequencing* (accession number)	*Rickettsia helvetica:* 23S PCR positives/ all tested (CT value)	*Other Rickettsia spp.:* gltA PCR positives/ all tested (CT value)
Melophagus ovinus	**81/81**	*Anaplasma ovis* (EF190509)	0/60	**1/60** (35)
Lipoptena cervi (free)[a]	**1/50**	*A. ovis* (EF190513)	0/50	0/50
L. cervi from red deer[a]	0/29	—	**3/29** (35–37)	**1/29** (41)
L. cervi from roe deer[a]	0/17	—	**3/17** (35–43)	**1/17** (29)

[a]Analyzed in pools.
PCR, polymerase chain reaction.
Cycle threshold (CT) is the number that PCR cycle in which fluorescent signal surpassed the baseline.
Bold values indicate sample groups in which positivity was found.

Anaplasma spp. that infect ruminants. Screening for Rickettsiaceae was done in two real-time TaqMan PCRs: one specific for *R. helvetica* (23S rRNA gene), and another to demonstrate all other *Rickettsia* spp. (*gltA* gene). Sequencing was attempted from samples with cycle threshold (CT) values below 30. All PCRs were run with appropriate controls. Extraction controls were negative, excluding cross-contamination of samples.

All sheep keds were found to harbor *A. ovis* that showed 100% sequence identity to a former Hungarian isolate (Table 1). This result may imply that each ked contained undigested blood. Although Zaugg and Coan (1986) failed to demonstrate the ability of sheep keds to transmit *A. ovis* between sheep, during their experiment it was not verified that these bacteria were actually present in the keds. Moreover, sheep keds are not host specific and will suck blood even on humans (Small 2005). Taking into account the report of Chochlakis et al. (2010) on the zoonotic potential of *A. ovis*, the present results suggest that the vector role of sheep keds for *A. ovis* needs to be evaluated in a broader context, including humans. In light of data in the literature, sheep keds may also be implicated in the epidemiology of *Bartonella melophagi*, a recently described human pathogen (Maggi et al. 2009).

Molecular identification of another *A. ovis* genotype in just one pool of free-living (winged) deer keds (Table 1) may indicate that only relevant flies have formerly sucked blood on infected hosts and/or reservoirs. Deer keds were reported to play a role in the transmission of *A. marginale* from wild to domestic ruminants (Drummond 1966). In addition, the present results show that deer keds are potential vectors for *A. ovis*. Taken together, finding *A. ovis* in both evaluated hippoboscid species may be relevant to the host range of *A. ovis*, which was recently suggested to be much broader than previously thought, including cattle and humans (Chochlakis et al. 2010, Hornok et al. 2010).

One sheep ked also contained a *Rickettsia* sp. (excluding *R. helvetica*) that could not be identified by sequencing (Table 1). This raises the possibility of rickettsaemia (*sensu stricto*) in sheep previously unreported in the literature. A similar reservoir role can be attributed to cattle as recently described (Jilintai et al. 2008). To the best of our knowledge, this is also the first report of rickettsiae in deer keds or any other hippoboscid flies. Whether or not they could transmit these agents—either during host shifts and regular blood meals or when becoming ingested (Small 2005)—remains to be clarified. Nevertheless, even tick-borne and flea-borne rickettsiae may have alternative modes of transmission (Sprong et al. 2009) that, according to the present results, may involve hippoboscid flies.

PCR positivity of keds from red deer and roe deer for *R. helvetica* (Table 1) also indicates that they sucked blood on infected hosts. Recently, roe deer were identified as reservoirs of *R. helvetica*, as confirmed by the present findings, but red deer was not proved to play such a role in the epidemiology (Sprong et al. 2009). Further, the relevant study (Sprong et al. 2009) failed to detect other *Rickettsia* spp. in either wild ruminants. Consequently, since here molecular evidence is provided for the occurrence of at least one more *Rickettsia* sp. in keds of both red deer and roe deer, the present results suggest a reservoir role for both wild ruminants concerning a broader range of rickettsioses.

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Disclosure Statement

No competing financial interests exist.

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